

## Genetic Relatedness and Population Structure in Primitively Eusocial Wasps in the Genus *Mischocyttarus* (Hymenoptera: Vespidae)

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*Abstract.*— Protein electrophoresis was used to investigate the genetic structure of two species of *Mischocyttarus*, a genus of primitively eusocial wasps. To do this we develop methods for estimating relatedness when there is population structure above the level of the colony. Relatedness among female colony-mates was quite high, consistent with the observations that only one or a few females had developed ovaries. One species, *M. basimacula*, showed no population structure above the colony level. The other, *M. immarginatus*, had a high inbreeding coefficient, which seems to arise from genetic differentiation of subpopulations that are less than 400 m apart. This subdivision means that individuals are quite closely related even to members of their own subpopulation who are not colony-mates. We discuss the possible relevance of these results to the evolution of the multiple-queen epiponine wasps.

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The polistine wasp genus *Mischocyttarus* occupies a special position for the study of the evolution of sociality in the Vespidae. It includes approximately 200 species, most of them in the neotropics but a few extending into Western North America and Florida (Richards 1978). *Mischocyttarus* is classified as primitively eusocial; although most individuals function as workers, there are no morphologically specialized castes. Since worker behavior is not pre-determined by morphology, primitively eusocial groups are especially suitable for studies of the selective advantage of worker behavior. Behavioral studies (Jeanne 1972; Litte 1977, 1979, 1981; Rodriguez 1989) have shown *Mischocyttarus* to be similar to the better-known genus *Polistes* in having a single queen who gains egg laying privileges by behaviorally dominating other females, although Itô (1984) has noted some tendency towards polygyny. However, *Mischocyttarus* is more interesting than *Polistes* in one respect, its phylogenetic position. It is more closely related (possibly the sister group) to an interesting and successful taxon of neo-tropical social wasps known as the Epiponini (Carpenter 1991, in press). The Epiponini are a clade of some 200 species that are socially more complex in several respects: (1) new colonies are founded by swarms of queens and workers, (2) colonies typically have multiple queens, and (3) castes are morphologically differentiated in some species (Richards 1978; Jeanne 1980, 1991). Since *Mischocyttarus* is closely related,

and possibly the sister group, studies of this genus might yield insights into the evolutionary history behind the traits that characterize the epiponines.

In this paper we focus on the genetic structure of two species of *Mischocyttarus* from the Yucatán peninsula of Mexico, *M. immarginatus* (Richards) and *M. basimacula* (Cameron). Hamilton (1964a, 1964b) showed that reproductively altruistic behavior such as that shown by social insect workers is promoted by high relatedness; genes for altruism will be lost unless the altruists aid individuals who share those genes. High relatedness is especially important in primitively eusocial species because workers are more likely to have the option of reproducing by themselves. Studies of primitively eusocial insects have generally confirmed this expectation (Metcalf and Whitt 1977; Lester and Selander 1981; Crozier et al. 1987; Schwarz 1987, 1988; Kukuk 1989; Ross and Matthews 1989; Strassmann et al. 1989). However, relatedness is usually lower within colonies of swarm-founding epiponines (Queller et al. 1988; West-Eberhard 1990; Strassmann et al. 1991) presumably because they often have several or many queens with developed eggs (Richards 1978; Jeanne 1980, 1991). It therefore seems possible that the same is true in their possible sister group, *Mischocyttarus*, particularly given Itô's (1984) finding (in two species, including *M. basimacula*) of multiple egg-laying females in the same colony. We have previously reported average relatedness among female colony-mates for *M.*

*immarginatus* and *M. basimacula* and found it to be high (Strassmann et al. 1989), which seems inconsistent with them having many queens.

Even if *Mischocyttarus* does not have multiple queens, it is still possible that it holds a different kind of key to understanding the evolution of multiple queens in its sister group. The relatedness-lowering effect of multiple queens might best be tolerated in social species that previously had very high relatedness. Inbreeding and population viscosity have been suggested as two population characteristics that might raise relatedness in this context (Hamilton 1972). If either of them operates in *Mischocyttarus*, then it may have also operated in the putative common ancestor of *Mischocyttarus* and the epiponines. In this report we focus on the effect of population structure above the colony level on genetic relatedness.

#### METHODS

Colonies, defined as a comb along with its resident adults, were collected in December 1987 during the dry season in Yucatán, México. Colonies of both species were located on buildings, usually on the underside of thatch eaves or in gaps within the thatch. Nineteen colonies of *Mischocyttarus immarginatus* were collected from three clusters located within 400 m of each other near the Uxmal archeological site. Three colonies of *M. basimacula* were collected from this site and 13 additional colonies were collected from 3 similarly close clusters near the Chichén Itzá archeological site, 130 km away. In both species, colonies within each cluster were no farther than 20 m from all other colonies in the cluster. All colonies within each cluster were collected, except as noted below, and we found no additional clusters in the areas between the collected ones. Our analyses will sometimes use these clusters of colonies as an additional level (which we will call the subpopulation level) at which interesting genetic structuring might occur. The clusters were not centered on colonies of more aggressive wasps, as has been reported for *M. immarginatus* (Windsor 1972).

Because some colonies were awkwardly positioned between layers of thatch, we were not always able to capture all of the adults. When any adults escaped, care was taken to see that they did not alight on neighboring nests. They did sometimes return to nearby abandoned nests, but did not generally alight on occupied nests before we could

collect them. On one or two occasions when this was uncertain, we did not collect the neighboring nest that might have been contaminated by foreign wasps.

The adults we captured were kept alive on ice for several days until they could be transferred to a low temperature (-70°C) freezer. Nests were scored for contents, stored on ice for several days, and then were kept in separate enclosures to allow pupae to emerge as adults. The 23 *M. immarginatus* females that emerged were deep frozen with the other adults and were later included in our analyses. The single female of *M. basimacula* that emerged was discarded.

The frozen adults were retrieved, dissected to determine ovarian condition, and subjected to protein electrophoresis. Standard starch gel methods, described in detail elsewhere (Strassmann et al. 1991) revealed three useful polymorphisms in *M. immarginatus* and two in *M. basimacula*. Males were scored as an aid in characterizing the Mendelian nature of the polymorphisms. However males were not included in the statistical analyses because they were few in number.

Relatedness estimates were obtained using the general method of Queller and Goodnight (1989). Below we extend the technique to fit populations that may be structured at the colony level and also at a higher subpopulations level. A brief description of the method is necessary to set the stage for extending it to sub-divided populations. For relatedness of one set of individuals,  $x$ , to another set,  $y$ , the basic estimator can be written as follows:

$$\frac{\sum_i \sum_k \sum_a w(p_{im} - p_m^*)}{\sum_i \sum_k \sum_a w(p_{im} - p_m^*)}$$

This is formula 10 of Queller and Goodnight (1989), with notation slightly altered. Summations are over all individuals of interest ( $i$ ), loci scored in that individual ( $k$ ), and the two (for diploids) allelic positions at that locus ( $a$ ). The  $p_{im}$ 's are various frequencies of the allelomorph currently being summed over (the one at position  $a$  of locus  $k$  of individual  $i$ );  $p_m$  is the frequency of that allele in individual  $i$  itself;  $p_m$  is the frequency of the set of  $i$ 's relatives whose relatedness is being estimated; and  $p_m^*$  is the frequency of the allele in the base population. The  $w$  is a statistical weight which can be chosen to give greater weight to certain individuals or to certain loci. This formula estimates the

relatedness coefficient proposed by Grafen (1985) and it is easy to see why it is a good descriptor of how selection works on behaviors that affect both an altruist and its colony members. The numerator totals up identities of potential altruist genes with genes of other colony members, and then subtracts the identities expected by chance, leaving an estimate of identity by descent. In the same way, the denominator estimates identities by descent of altruist's genes with their own genotypes. The ratio therefore describes the relative value of the altruist and of other colony members as vehicles for the propagation of the altruist's genes.

The asterisk of  $p_m^*$  serves to indicate a correction necessary to remove a bias present in earlier relatedness measures (Pamilo and Crozier 1982; Pamilo 1984, 1989). To get an unbiased estimate of the differences in the numerator and the denominator, it is necessary that the first and second terms of these differences be estimated independently (Queller and Goodnight 1989). How this is accomplished is best illustrated by example. Suppose we have a population subdivided into colonies, indexed by values of  $c$ , and we want to estimate average relatedness to colony mates. Formula 1 can be written as:

$$r = \frac{\sum_i \sum_k \sum_a w(p_{ci-im} - p_{c-im})}{\sum_i \sum_k \sum_a w(p_{im} - p_{c-im})}$$

Here the set of relatives of interest is all colony mates except for the individual itself, so the frequency of allelomorph  $m$  in these relatives is written as  $p_{ci-im}$ . The population frequency of this allele, estimated to avoid bias, is written as  $p_{c-im}$ , that is, the population frequency is estimated after excluding the current colony. This means that the estimate of the population frequency changes slightly according to which colony the potential altruist belongs to. This step is necessary whenever we have a small sample of colonies drawn from a much larger population. Including the colony in the estimate would lead to an overestimation of its contribution to the population frequency and therefore an underestimation of the difference from the colony frequency.

When there is population structure above the level of the colony (sub-populations or demes), then several different  $r$ 's may be of interest. Pamilo (1984, 1989) gave these names analogous to F-statistics.

The relatedness to colony mates with respect to the total population is called  $r_{ct}$ . This is essentially the measure described above, although it should be estimated slightly differently when there is sub-population structure (see below). Relatedness to members of the whole sub-population (other than one's own colony) is called  $r_{st}$ . It would be relevant to describing selection on a broader kind of altruism that extends beyond the confines of the colony to the whole sub-population. Finally  $r_{cs}$  is the relatedness of colony members with respect to their own sub-population rather than with respect to the whole population. This relatedness is the one that is most relevant to the spread of colony altruism alleles within isolated sub-populations that are evolving relatively independently.

Table 1. Estimation of relatedness. Table entries are the frequencies of allelomorph  $m$  required for using equation 1 to estimate various measures of relatedness. The first  $r$  is for estimation under the assumption of no population subdivision above the colony level. The others estimate relatedness within colonies with respect to the total population, within colonies with respect to their own subpopulation, and within subpopulations with respect to the total population.

relatedness measure	frequency in relatives ( $p_{im}$ )	frequency in population ( $p_m^*$ )
$r$	$p_{ci-im}$	$p_{c-im}$
$r_{ct}$	$p_{ci-im}$	$p_{c-im}$
$r_{cs}$	$p_{ci-im}$	$p_{st-cim}$
$r_{st}$	$p_{st-cim}$	$p_{s-im}$

Estimates for these three relatedness parameters are obtained using formula 1, with the substitutions indicated in Table 1. The estimate for  $r_{ct}$  is the same as that for  $r$  without population structure (formula 2) except that the estimate of population frequency necessary to avoid bias is slightly different. Now, this frequency should be calculated excluding the whole sub-population ( $p_{c-im}$ ) instead of just the colony. The same procedure is required for  $r_{st}$ . The corresponding measure for  $r_{cs}$  is the frequency in the sub-population, after having excluded the current colony ( $p_{st-cim}$ ). The only change is that for  $r_{st}$ , the set of relatives of interest is the subpopulation (except members of the individual's

own colony).

These statistics can be related to each other by a formula analogous to one well-known for F-statistics:  $1 - r_{st} = (1 - r_{ci})(1 - r_{st})$  (Pamilo 1984). The estimators of these statistics developed here also possess this property.

F-statistics are estimated in analogous fashion (Queller and Goodnight 1989, equations 13-15). In the absence of sub-population structure, the inbreeding coefficient can be estimated as

$$f = \frac{\sum_i \sum_k \sum_a w(p_{i(a)m} - p_{(c)m})}{\sum_i \sum_k \sum_a w(1 - p_{(c)m})}$$

where  $p_{i(a)m}$  is the frequency of allelomorph  $m$  in the individual  $i$ ,  $i$ , currently being summed over, but not including the allelic position,  $a$ , currently being summed over. In other words, it is the frequency at the other allelic position. Other than changes in notation, this differs from the estimate for  $F_{it}$  (Queller and Goodnight 1989, equation 14) only by using  $p_{(c)m}$  instead of  $p_{(c)m}$  as the estimate of the population frequency. This is exactly analogous to the already-noted difference between  $r$  and  $r_{ci}$ .

For all estimates in this report we weight colonies equally, so  $w$  is set equal to the reciprocal of the number of females scored. To obtain standard errors for most estimates, we jackknife (Sokal and Rohlf 1981) over colonies, which simulations show to be a satisfactory procedure (Queller and Goodnight 1989). The exceptions are the sub-population parameters,  $r_{st}$  and  $F_{st}$ , which are jackknifed over sub-populations (because there are few sub-populations, these estimates are less reliable). For both relatedness and F-statistics, the jackknife standard errors can be used to construct confidence intervals or to conduct t-tests using t distributions with degrees of freedom equal to one less than the number of colonies (or subpopulations for  $r_{st}$  and  $F_{st}$ ) (Sokal and Rohlf 1981).

## RESULTS

Table 2 shows characteristics of the colonies of both species. Both were raising brood during the dry season, although some colonies, especially of *M. basimaculata*, had little or no brood. On average, *M. basimaculata* colonies were raising fewer brood than *M. immarginatus*, as can be seen most clearly from the difference in the percent of cells containing pupae or large larvae. In each species there were some new nests, identified by the absence of

Table 2. Colony characteristics of *Mischocyttarus* species (means  $\pm$  standard deviations).

	<i>M. basimaculata</i>	<i>M. immarginatus</i>
cells	61.5 $\pm$ 42.1	54.5 $\pm$ 51.8
pupae	4.2 $\pm$ 3.6	9.3 $\pm$ 9.7
large larvae	2.8 $\pm$ 2.6	5.9 $\pm$ 5.2
(pupae + large larvae) / cells	0.17 $\pm$ 0.12	0.33 $\pm$ 0.16
adults with layable eggs *	2.44 $\pm$ 1.96	2.18 $\pm$ 2.37

\*Since some adults escaped capture, this is an underestimate.

pupae and of cells bearing the remnants of pupal cocoons.

In each species the arithmetic mean number of females with layable eggs per colony exceeded two (Table 2). The modal number was one. If all the females with layable eggs are actually successful at laying them and having them raised, then this should tend to lower relatedness within colonies. However, Wade (1985) has shown that the correct measure for considerations of the effect of queen number on average relatedness is the harmonic mean number of egg layers (this is because relatedness is a function of the reciprocal of queen number, and the harmonic mean involves the mean of reciprocals). The harmonic mean number of egg layers is 1.54 for *M. basimaculata* and 1.27 for *M. immarginatus* (for this measure, colonies in which no dissected females had layable eggs were assigned as having one egg-layer). It should be remembered that these numbers are probably slightly lower than the actual numbers since some females escaped capture.

Tables 3 and 4 list the allele frequencies for the polymorphic loci. The estimates of relatedness and inbreeding, assuming no population structure above the level of the colony, are given in Table 5. Female colony mates in both species are highly related, with the value for *M. immarginatus* being particularly high and very close to that expected for the progeny of one singly-mated outbred female (0.75). However, *M. immarginatus* does not fit this model because it is highly inbred. There is no evidence for inbreeding in *M. basimaculata*.

Estimates of F-statistics, reported in Table 6, can reveal some additional aspects of population struc-

Table 3. Allele frequencies in *Mischocyttarus basimacula*. GPI = glucose-phosphate isomerase; PEP = peptidase. All frequencies are calculated by giving each colony equal weight.

	# colonies	# individuals	GPI		PEP	
			a	b	a	b
whole population	16	75	0.57	0.43	0.98	0.02
subpopulation 1	3	24	0.57	0.43	1.00	0.00
subpopulation 2	5	21	0.58	0.42	0.96	0.04
subpopulation 3	5	21	0.60	0.40	0.97	0.03
subpopulation 4	3	9	0.51	0.49	1.00	0.00

Table 4. Allele frequencies in *Mischocyttarus immarginatus*. PGD = 6-phosphogluconate dehydrogenase; PGM = phosphoglucomutase; GDA = guanine deaminase. All frequencies are calculated by giving each colony equal weight.

	# colon- nies	# individuals	PGD		PGM		GDA	
			a	b	a	b	a	b
whole pop.	19	125	0.89	0.11	0.38	0.62	0.62	0.38
subpop. 1	3	6	0.58	0.42	0.00	1.00	0.33	0.67
subpop. 2	12	85	0.96	0.04	0.56	0.44	0.85	0.15
subpop. 3	4	34	0.91	0.09	0.12	0.88	0.13	0.87

Table 5. Within-colony relatedness and inbreeding coefficients for adult females. Estimates are given with standard errors. Brackets enclose 95% confidence intervals.

Species	Relatedness ( <i>r</i> )	Inbreeding ( <i>f</i> )
<i>M. basimacula</i>	0.435 ± 0.116 [0.186 to 0.683]	-0.105 ± 0.140 [-0.404 to 0.193]
<i>M. immarginatus</i>	0.766 ± 0.036 [0.691 to 0.841]	0.367 ± 0.066 [0.229 to 0.505]

Table 6. F-statistics for *Mischocyttarus*. Starred values are significantly greater than zero (one-tailed t-test). The subpopulations are clusters of nests usually located within 400 m of each other (see text).

species	statistic estimated	estimate ± s.e.	95%
			confidence interval
<i>M. basimacula</i>	$F_{st}$	-0.126 ± 0.133	-0.408 to 0.156
	$F_{is}$	-0.066 ± 0.016	-0.118 to -0.014
	$F_{is}$	-0.056 ± 0.160	-0.396 to 0.284
<i>M. immarginatus</i>	$F_{st}$	0.491 ± 0.089*	0.305 to 0.678
	$F_{st}$	0.437 ± 0.193	-0.392 to 1.000
	$F_{is}$	0.097 ± 0.143	-0.204 to 0.398

Table 7. Relatedness under the assumption of population subdivision. Starred values are significantly greater than zero (one-tailed t-test)

species	relatedness type	<i>r</i> ± s.e.	95%
			confidence interval
<i>M. basimacula</i>	$r_{ct}$	0.411 ± 0.114*	0.167 to 0.654
	$r_{cs}$	0.488 ± 0.121*	0.230 to 0.749
	$r_{st}$	-0.151 ± 0.061	-0.344 to 0.042
<i>M. immarginatus</i>	$r_{ct}$	0.828 ± 0.040*	0.745 to 0.911
	$r_{cs}$	0.584 ± 0.115 *	0.344 to 0.825
	$r_{st}$	0.586 ± 0.164 *	-0.118 to 1.000

ture. The point estimates of the F-statistics for *M. basimacula* are all slightly negative. The 95% confidence intervals for  $F_{st}$  and  $F_{is}$  show them to be consistent with zero values. Strangely, though the point estimate of  $F_{st}$  is quite close to zero, the confidence interval falls entirely below this value, suggesting that individuals tend to be slightly less similar to members of their own subpopulation (outside of their own colony) than they are to members of other subpopulations.

The inbreeding in *M. immarginatus* (high  $F_{st}$ ) seems to be due to population subdivision (high  $F_{st}$ ) with little contribution from inbreeding within the subpopulations (low  $F_{is}$ ). This conclusion is not certain because while the point estimate of  $F_{st}$  is quite high, its 95% confidence interval includes zero. If

we conduct a one-tailed t-test of the reasonable a priori hypothesis that  $F_{st}$  is greater than zero, it is nearly significant ( $0.05 < p < 0.1$ ).

Relatedness values are consistent with these findings (Table 7). Since there is little structure at the subpopulation level in *M. basimacula*, it is not surprising that relatedness to colony mates does not change much when estimated with respect to the subpopulation ( $r_{cs}$ ) instead of with respect to the whole population ( $r_{ct}$ ). Similarly, we should not expect individuals to be significantly related to subpopulation members other than their colony-mates ( $r_{cs}$ ), and they are not.

More interesting results are expected for *M. immarginatus* since it does appear to be structured at the subpopulation level. Since some of the similarity within colonies can be attributed to a general similarity within subpopulations, relatedness within colonies is considerably lower when measured with respect to the subpopulation ( $r_{cs} = 0.58$ ) than it is when estimated with respect to the whole population ( $r_{ct} = 0.83$ ). Moreover, individuals appear to be closely related to members of their own subpopulation who are not colony-mates ( $r_{st} = 0.59$ ). This value is significantly greater than zero (one-tailed t-test).

Some observations bearing on this point were obtained during collection. Small active nests were sometimes located close to large and often inactive nests. Adults that we missed when collecting from active colonies would sometimes return to the inactive nest. This might indicate a general tendency of adults to move among nearby nests. Alternatively, it might be an indication of a specific past relationship with the abandoned colony, perhaps as a parent-colony.

#### DISCUSSION

Relatedness within colonies is fairly high in these two species of *Mischocyttarus*, in general agreement with results from *Polistes* (Metcalf and Whitt 1977, Lester and Selander 1981, Strassmann et al. 1989) and other primitively eusocial insects (Crozier et al. 1987; Schwarz 1987, 1988; Kukuk 1989; Ross and Matthews 1989). This means that worker behavior can be favored by kin selection without requiring extraordinarily high benefit-cost ratios (Hamilton 1964a,b, 1972). *M. basimacula* is in the low end of the range, and would therefore require a somewhat higher benefit-cost ratio than *M. immarginatus*, which is one of the species with highest relatedness, very near the outbred full-

sister value of  $3/4$ .

The two species differ markedly with respect to the presence of population substructure above the colony level, and this may have some consequences for relatedness. *M. basimacula* lacks both inbreeding and subpopulation differentiation. In fact the  $F_{st}$  is significantly negative, suggesting that a randomly chosen individual is more similar to members of other subpopulations than it is to members of its own subpopulation (excluding its own colony). We are unaware of any population process that might be operating to produce such an effect and suspect that this is a sampling effect; in a population with no substructure 1 in 20 samples will give a "significantly" negative  $F_{st}$ . In any event, the estimate is close enough to zero so that there is little effect on relatedness (compare  $r_{cs}$  and  $r_{st}$ ).

*M. immarginatus* does show some pronounced population structure. The population taken as a whole is quite highly inbred.  $F_{st}$  and  $f$  are high and significantly greater than zero (these two measures estimate essentially the same thing but differ because the latter is estimated under the assumption that there is structure at the subpopulation level). Since  $1 - F_{st} = (1 - F_{cs})(1 - F_{st})$ , the inbreeding at the population level ought to be attributable to either population subdivision or to inbreeding within the subpopulations. Curiously, neither  $F_{st}$  nor  $F_{cs}$  is significantly different from zero, but the much higher point estimate for  $F_{st}$  suggests that population subdivision is responsible for the apparent inbreeding at the population level. Subpopulation structure has been detected in some other primitively eusocial insects. A very modest amount of differentiation has been found in a halictid bee (Crozier et al. 1987), a sphecid wasp (Ross and Matthews 1989) and an anthophorid bee (Blows and Schwarz, pers. comm.). Highly differentiated subpopulations (separated by several kilometers) have been found only in *Polistes exclamans* (Viereck) (Davis et al. 1990). Three other *Polistes* species investigated in the same study showed no such differentiation.

Can *Mischocyttarus* tell us anything about the evolution of social traits like polygyny in the Epiponini? Itô (1984) has argued that some members of the *Mischocyttarus*, including *M. basimacula*, are polygynous. If this were true, *Mischocyttarus* and the Epiponini might have inherited the polygynous habit from a common ancestor. However, our study has revealed little evidence for true polygyny in *Mischocyttarus*. Some colonies had

more than one female with developed eggs, but the harmonic mean number of egg-layers was quite low. Moreover, relatedness in both species was too high to allow for very many egg-layers. Our data tend to support the conventional view that one female is usually able to dominate the others (Jeanne 1972; Litte 1977, 1979, 1981), perhaps with a minor amount of egg laying by subordinates.

In a different way, *M. immarginatus* seems to have a kind of population structure that could make it a suitable model for a species ancestral to the polygynous Epinonini. Polygyny tends to lower relatedness within colonies, which should increase selection for selfish behavior and could therefore erode the basis which maintains the structure of social insect colonies. This problem would be least likely to occur when polygyny arises in a species with initially high relatedness, due to single mating, inbreeding, or population viscosity. Of course this is not a barrier preventing polygyny from arising in species with low relatedness, but it could pose a barrier to the continued maintenance of worker behavior in such species. It should be noted that while *M. immarginatus* seems to have this suitable kind of population structure, two findings argue against the hypothesis. First, *M. basimaculata* does not have this kind of population structure and we do not know which species is more representative of the ancestor to the epinonines. Second, the three epinonine species that have been studied genetically show no inbreeding (Queller et al. 1988). These species were collected from areas as large as, or larger than, the area from which we collected *M. immarginatus*. Therefore, at least on this spatial scale, there seems to be no population subdivision in these three epinonines, and positing an ancestral population structure like that of *M. immarginatus* seems unnecessary. Further studies of *Mischocyttarus* and of additional epinonines might alter this conclusion.

The population subdivision in *M. immarginatus* makes the interpretation of relatedness coefficients more interesting and more complicated. If the subpopulations are reproductively isolated, then  $r_{st}$  is the appropriate measure for predicting the average spread of altruism alleles within the subpopulations. The total population gene frequency does not enter into this measure if members of different subpopulations do not compete reproductively. However, this assumption seems unlikely. Although we have no direct evidence, it seems improbable that these subpopulations,

separated by less than 400 m, could be completely isolated.

If we assume that the subpopulations are not reproductively isolated, then  $r_{st}$  may be the more appropriate measure for understanding the evolution of altruism among colony mates. Similarly  $r_{st}$  would be relevant to the evolution of altruism towards other members of the same subpopulation. However, three caveats must be added. First, since there is nonrandom mating, relatedness coefficients are exact only for social traits genes with additive dosage (Michod and Hamilton 1980; Seger 1981, Grafen 1985). Second, if the local subpopulation sizes are regulated independently, then success within subpopulations may translate nonlinearly into success in the population as a whole. This is a special case of non-additive fitness components, a phenomenon that can make inclusive fitness models inexact (Cavalli-Sforza and Feldman 1978; Queller 1985). Finally, another complication arises if the similarity within subpopulations arises from pedigree connections more than a few generations back (Grafen 1985) because any new altruism allele would not experience the same structure shown by the marker alleles. However, this may not apply to *M. immarginatus*. Because the subpopulations are so close together, it seems most likely that the *M. immarginatus* subpopulations have been separated (probably partially) for only a very short time.

These caveats aside, the  $r_{st}$  estimate shows that relatedness to female colony-mates is very high, closely approximating the full-sister value of 3/4. Relatedness to other members of the same subpopulation ( $r_{st}$ ) is also quite high, so altruism towards individuals in other colonies could be favored. Whether such altruism occurs is unknown. Wasps that we had disturbed sometimes returned to other nests, suggesting some sort of connection between nests, but the new nests were usually abandoned or inactive.

The exact reason for the population structure is unknown. The simplest explanation is a strong tendency to begin new nests close to the natal nest. Several different patterns of this type have been reported from primitively eusocial polistine wasps. In *Polistes canadensis* (L.), multiple combs are constructed as part of a single colony with a single queen, and there is fluid movement of individuals among the combs (Jeanne 1979). This differs from *M. immarginatus* for several reasons. First, each comb had at least one female with developed ova-

ries. Second, having a single queen and fluid movement among nests should produce the same degree of relatedness between combmates and non-combmates, but we found the former to be higher. Finally, the combs in clusters of *M. immarginatus* were usually separated by at least 15 cm, compared to less than 3 cm in *Polistes canadensis*.

A more likely pattern that could lead to subdivision is the formation of satellite nests. In *Polistes exclamans*, females from an established nest may begin a new nest nearby, but connections between the parent and daughter nest are relatively ephemeral: movement of workers between them decreases and each has its own queen (Strassmann 1981a,b). Alternatively, simple philopatry may lead to the establishment of new nests near the site of the old inactive parent nest. This common pattern (Noonan 1981; Strassmann 1983) may also occur in *M. immarginatus* at another site in Mexico (Rodriguez 1989).

#### ACKNOWLEDGMENTS

We thank J. M. Carpenter for identifying the species and W.P. and E.M. Strassmann for help in the field. Keith F. Goodnight wrote the computer program that performed most of the calculations. Voucher specimens have been deposited with Universidad Nacional Autónoma de México. Funded by NSF grants BSR 8605026, BSR 8805915 and BSR 9021514.

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